Title: Scarless removal of large resistance island AbaR results in antibiotic susceptibility and increased natural transformability in *Acinetobacter baumannii.*

Running title: Scarless removal of AbaR

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ABSTRACT

With a great diversity in gene composition including multiple putative antibiotic-resistance genes, AbaR islands are potential contributors to multi-drug resistance in *Acinetobacter baumannii*. However, the effective contribution of AbaR to antibiotic resistance and bacterial physiology remains elusive. To address this, we sought to accurately remove AbaR islands and restore the integrity of their insertion site. To this end, we devised a versatile scarless genome editing strategy. We performed this genetic modification in two recent *A. baumannii* clinical strains: the strain AB5075 and the nosocomial strain AYE which carry AbaR11 and AbaR1 islands of 19.7 kbp and 86.2 kbp, respectively. Antibiotic susceptibilities were then compared between the parental strains and their AbaR-cured derivatives. As anticipated by the predicted function of the ORF of this island, the antibiotic resistance profiles were identical between the wild type and the AbaR11-cured AB5075 strains. In contrast, AbaR1 carries 25 ORFs with a predicted resistance to several classes of antibiotics and the AYE AbaR1-cured derivative showed restored susceptibility to multiple classes of antibiotics. Moreover, curing of AbaRs restored high levels of natural transformability. Indeed, most AbaR islands are inserted into the *comM* gene involved in natural transformation. Our data indicate that AbaR insertion effectively inactivates *comM* and that the restored *comM* is functional. Curing of AbaR consistently resulted in highly transformable, and therefore, easily genetically tractable strains. Emendation of AbaR provides insight into the functional consequences of AbaR acquisition.

INTRODUCTION

Acinetobacter baumannii is responsible for healthcare-associated infections and concerns because of its intrinsic resistance to many antimicrobials and its ability to acquire resistance genes (1,2). A potential contributor to multi-drug resistance in *A. baumannii* is a genomic island, named AbaR, with a great diversity in gene content and carrying multiple putative antibiotic-resistance genes (3,4). The first report of AbaR described a large genomic island of 86 kbp in the epidemic *A. baumannii* strain AYE in 2006 (5). Since then, analysis of more than 3000 *A. baumannii* genomes deposited in the NCBI database revealed that AbaR are present in nearly 65% of them (4). With a transposon backbone, AbaR islands are considered as mobile genetic elements and present a modular structure (3,6–8). Although AbaR may contain up to 25 putative antibiotic-resistance genes, most AbaR carry three to four genes predicted to confer resistance mainly to aminoglycosides and tetracycline and with a median

65 length of 20 kbp (4,9). Despite increasing genomic data, the actual contribution of AbaR-type
66 islands to antibiotic resistance has not been fully evaluated. Deletion mutants of the AbaR23
67 island were previously obtained and suggested a paradoxical role of AbaR in increasing
68 susceptibility to ciprofloxacin (9). However, deletions of AbaR23 was obtained by allelic
69 replacement of AbaR23 with the *aacC1* cassette conferring resistance to gentamicin and
70 confounded susceptibility testing against aminoglycosides. In addition, the original insertion
71 site of AbaR was not restored which prevented analysis of the physiological consequence of
72 the insertion of AbaR in the targeted gene. AbaR islands have been found to insert into 51
73 chromosome-borne loci, yet the overwhelming majority of AbaR islands are found inserted in
74 the *comM* gene (4). This later gene encodes for a putative ATPase involved in natural
75 transformation in a few transformable species including non-pathogenic *Acinetobacter baylyi*
76 (10,11). Natural transformation allows bacteria to actively import exogenous DNA and, if the
77 sequence of the imported DNA presents sufficient identity, it integrates the recipient cell's
78 chromosome by homologous recombination (12,13). Interestingly, the *comM* gene is not
79 strictly essential for transformation but its mutation decreases the transformation efficiency by
80 two orders of magnitude (10,11). *A. baumannii* and related species *A. nosocomialis* were
81 described as transformable in 2013 (14,15). In *A. baumannii*, natural transformation appears
82 as a conserved trait among clinical and non-clinical isolates of human and animal origin
83 (14,16), providing the bacteria with a major route for acquisition of antibiotic resistance genes.
84 In this study, we aim at assessing the contribution of AbaR islands to antibiotic resistance of
85 two human clinical strains of the main Global Clone 1 bearing small and large AbaRs. We
86 also analyzed the consequences of AbaR-mediated *comM* gene disruption on the
87 transformation efficiency of these strains. We bring evidence that interruption of the *comM*
88 gene by AbaR results in a decrease in natural transformation. Using genetic approaches, we
89 further confirm a role for the *comM* gene in natural transformation in *A. baumannii*.
90

91 **MATERIALS AND METHODS**

92 *Bacterial strains, typing and growth conditions*

93 The bacterial strains used in this study are listed in Table S1. Unless specified, *A. baumannii*
94 isolates and strains were grown in lysogeny broth (LB) (Lennox). All experiments were
95 performed at 37°C. Antibiotic concentrations in selective media were 30 µg/mL for apramycin
96 and 100 µg/mL for rifampicin.
97

98 *Construction of bacterial strains*

99 All oligonucleotides used in this study for genetic modification are listed in supplementary
100 Table S2. Gene disruptions were performed using overlap extension PCR to synthesize large
101 chimeric DNA fragments carrying the selection marker flanked by 2 kbp-long fragments that
102 are homologous to sequence flanking the insertion site. The PCRs were performed with a
103 high-fidelity DNA polymerase (PrimeStarMax, Takara) (see section *Detailed protocol for*
104 *chimeric PCR for mutation in A. baumannii* in the supplemental material) . When used as
105 template for the generation of PCR products, genomic DNA was extracted and purified using
106 the Wizard® Genomic DNA purification kit (Promega) following the manufacturer's guidelines.
107 The AB5075 wild type strain was naturally transformed with an assembly PCR product to
108 generate the AB5075 *comM::sacB_aac* strain. The AYE *comM::sacB_aac* was then obtained
109 by transforming the AYE wild type strain with genomic DNA extracted from the AB5075
110 *comM::sacB_aac* strain. For both strains, transformants were selected on LB plates
111 supplemented with apramycin and susceptibility to sucrose was verified on M63 plates
112 supplemented with 10% sucrose. The AB5075 and AYE *comM::sacB_aac* strains were
113 naturally transformed with an assembly PCR product (obtained from assembly PCR on wild
114 type genomic DNA) to generate their -T and Δ*comM* derivatives. Transformants were
115 selected on M63 plates supplemented with 10% sucrose and susceptibility to apramycin was
116 verified on LB plates supplemented with apramycin. All chromosomal modifications were
117 verified in transformants using colony PCR (cf. Table S2). Removal of AbaR and restoration
118 of the *comM* gene were verified by Sanger sequencing.

119 The AB5075 rifampicin resistant (RifR) strain was generated by selecting spontaneous
120 mutants after plating an overnight culture of the AB5075 wild type strain on plates
121 supplemented with rifampicin.

122

123 *Antibiotic susceptibilities testing*

124 Susceptibility of the strains to a panel of 15 antibiotics (ticarcillin, ticarcillin-clavulanic acid,
125 piperacillin, piperacillin-tazobactam, ceftazidime, cefepime, meropenem, imipenem,
126 gentamicin, tobramycin, amikacin, ciprofloxacin, tetracycline, aztreonam and sulfonamide)
127 was evaluated by disc diffusion on Muller-Hinton agar (Biorad, France) following the CA-SFM
128 2013 recommendations

129 (https://resapath.anses.fr/resapath_uploadfiles/files/Documents/2013_CASFM.pdf). Inhibition
130 values were interpreted according to CA-SFM 2013 breakpoints for all antibiotics but
131 aztreonam, for which *Pseudomonas* spp. breakpoints are given. To compare tetracycline
132 susceptibility (as resistance marker of AbaR1) susceptibilities of wild type AYE and deleted
133 AbaR1 mutant were compared by E-test (bioMérieux, France). The strain *Pseudomonas*
134 *aeruginosa* CIP 7110 was used as control.
135
136 *Transformation assay*
137 The method used in this study was described in (16) with the following modifications. After
138 overnight incubation at 37°C on Lennox Broth agar, the strains were cultured for a few hours
139 in 2 mL of Lennox Broth (LB) until an OD600nm of at least 1. The bacterial broths were then
140 diluted to an OD600nm of 0.01 in PBS. Then, small aliquots of bacterial suspensions (5-10 µl)
141 were mixed with DNA substrate. To measure transformation frequency, a final PCR
142 concentration of 50 ng/µl was used. The mixtures (2.5 µl) were deposited on the surface of 1
143 ml of transformation medium (2% agarose D3 (Euromexdex), 5g/L of tryptone, 2,5g/L NaCl)
144 /poured in 2-mL micro tubes and incubated overnight at 37°C. The next day, bacteria were
145 recovered by resuspension in 300 µl of PBS. For genome edition experiments, the
146 suspensions were spread on selective media (see *Construction of bacterial strains* section for
147 details). To measure transformation frequency the bacterial suspensions were serial diluted
148 and spread on LB agar plate without antibiotic or supplemented with rifampicin. All the
149 transformation assays were performed on at least two separate experiments. On each
150 occasion, at least three independent transformation reactions were conducted (three different
151 transformation tubes). All the independent data points are plotted. As normality of the
152 distribution of transformation frequency does not apply for transformation frequency analysis,
153 non-parametric tests were performed (Mann-Whitney-Wilcoxon).
154
155 **RESULTS AND DISCUSSION**
156 ***Curing AbaR in A. baumannii using a scarless genome editing strategy***
157 In order to cure *A. baumannii* of the AbaR island while restoring the original insertion site, we
158 devised a scarless genome editing strategy. In contrast to other methods, the method does
159 not require cloning, or prior genetic engineering of the target strain (17–19). Rather, it relies
160 on the use of chimeric PCR products and takes advantage of natural transformation, a
161 phenotypic trait exhibited by most isolates of *A. baumannii*. The method is based on a two-

162 step selection (Fig. 1), first the introduction of a selectable/counter-selectable *sacB_aac*
163 cassette at the loci of interest (Fig. 1B), then the replacement of the cassette by the desired
164 sequence (Fig. 1C). First, a chimeric PCR product is assembled, consisting of the *sacB_aac*
165 cassette bearing a selection marker (*aac(3)IV)* conferring resistance to apramycin, flanked by
166 2 kb-long sequences identical to the target region (Fig. 1B). This chimeric PCR product is
167 introduced by natural transformation and *A. baumannii* transformants are selected using
168 apramycin, an antibiotic to which most clinical isolates are sensitive. A second PCR product is
169 assembled, still carrying the homologous flanking regions but whose sequence is designed to
170 produce deletion, insertion or single-base mutation (deletion in Fig.1C). Genetic replacement
171 and the loss of previously inserted *sacB* gene is then selected using sucrose resistance
172 selection. We exemplified the method by deleting AbaR11 in strain AB5075. The strain
173 AB5075 is an *A. baumannii* ST1 strain isolated from a bone infection in 2008 with multidrug
174 resistance to eight antibiotic classes (aminoglycosides, fluoroquinolones, beta-lactams
175 including penicillins, third and fourth generation cephalosporins, and carbapenems) (20). This
176 strain bears a 19.7 kbp-long AbaR11 inserted in the *comM* gene with 19 predicted genes
177 corresponding to five modules found in other AbaRs (Fig. 2A) (9,21,22). In a previous study,
178 we identified the strain AB5075 as naturally transformable (16). Using natural transformation
179 of PCR chimeric products, the AbaR island was replaced upon genetic recombination by the
180 *sacB_aac* cassette (Fig. 2B). Then, the strain bearing the *sacB_aac* cassette in the *comM*
181 gene was subjected to transformation with an assembly PCR encompassing the insertion
182 region cured of AbaR thereby carrying an intact *comM* gene. Then we performed genetic
183 analysis of sucrose-resistant and apramycin-susceptible clones to select an AbaR-cured
184 strain (named AB5075-R) with a restored *comM* gene presumably encoding a full-length
185 ComM protein (Fig. 2C).
186
187 ***Resistance profiles associated with AbaR curing in two clinically relevant MDR strains***
188 We then sought to investigate the role of AbaR island in the multi-drug resistance profile of
189 strain AB5075. With 19.7 kbp, AbaR11 falls in the median size of AbaRs (4). This AbaR
190 contains 19 ORFs with, besides transposon-related proteins, seven ORFs predicted to confer
191 resistance to metals, three of unknown function and one (*sup*) related to sulfonamide
192 resistance encoding a putative sulfate permease present in the K-module found in most
193 AbaRs (Fig. 2A). Antibiotic susceptibilities were thus compared between the AB5075 parental
194 strain and its AbaR-cured derivative, denoted AB5075-T (Table 1). As anticipated, the

195 resistance profiles to 15 antibiotics belonging to five antibiotic classes were identical between
196 the wild type and its AbaR-cured derivative including to sulfonamides. We then investigated
197 the contribution to antibiotic resistance of a larger AbaR found only in another *A. baumannii*
198 ST1 strain, the clinical strain AYE resistant to aminoglycosides, ß-lactams (excluding
199 imipenem, piperacillin-tazobactam, and ticarcillin-clavulanate), fluoroquinolones, and
200 tetracycline and was of intermediate susceptibility to rifampicin (5,23). This strain carries the
201 largest AbaR described so far, AbaR1, with 86 kbp-long, containing 25 genes putatively
202 involved in resistance to antibiotic of several classes. By exploiting natural transformation of
203 the strain AYE (16), we cured this strain of its AbaR1 island and compared the resistance
204 profiles of the parental strain and its AbaR-cured AYE-T derivative (Table 1). In contrast to
205 strain AB5075, curing the AbaR island from the strain AYE resulted restored susceptibility to
206 aminoglycosides, tetracycline, sulfonamide and to some ß-lactams. A partial decreased
207 resistance to ticarcillin (carboxypenicillin), ceftazidime (3GC), cefepime (4GC), aztreonam
208 (monobactam) was also observed. The resistance *armamentarium* conferred by the 86 kbp-
209 long AbaR1 may be attributed to the *aadB*, *aac6*, *aac3* and *aadDA1* genes for the resistance
210 to amikacin, tobramycin and gentamicin (aminoglycosides), to the two *tetA-R* genes for
211 tetracycline resistance and, to five copies of the *sulI* gene for the sulfonamide resistance (5).
212 Partial loss in resistance to ticarcillin, ceftazidime, cefepime and aztreonam may be mainly
213 attributed to loss of the bla_{VEB-1} gene and to a lesser extent the bla_{oxa-10} gene, this latter
214 conferring resistance to all ß-lactams but carbapenems and extended spectrum
215 cephalosporins (5,23). Only a partial loss of resistance to beta-lactams upon AbaR1 curing is
216 likely explained by the additional resistance mechanisms of the AYE strain possess, in
217 particular the over-expression of the bla_{ADC} chromosomal cephalosporinase or from other
218 genomic locations with the prediction of a new class A ß-lactamase on AYE chromosome
219 (5,24). Contrarily to a previous report, we did not observe for both strains an increased
220 resistance to fluoroquinolone of the AbaR-cured strains (9).
221
222 ***Curing of AbaR and repair of the comM gene result in increased transformation ability***
223 Among the 51 chromosome-borne insertion loci, AbaR islands are predominantly inserted in
224 the *comM* gene (4). In the non-pathogenic species *A. baylyi*, a mutation of this latter gene is
225 associated with a reduced transformability (10). We therefore pursued by investigating the
226 role of *comM* in *A. baumannii* and the physiological consequence of AbaR-island disruption of
227 this gene. To do so, we performed transformation assays comparing the levels of

228 transformation efficiency between the wild type and AbaR-cured strains (Fig. 3). For both
229 clinical strains, curing AbaR and thus restoring the *comM* gene increased significantly the
230 ability to acquire a genetic marker with a mean increase of about a 1000-fold in strain
231 AB5075-T (Fig. 3A) and of about 300-fold in strain AYE-T (Fig. 3B). With one bacterium out of
232 100 that performed natural transformation, strain AB5075-T presented the highest
233 transformation ability (Fig. 3A). Using the same genome-editing strategy as depicted in Fig. 1,
234 we generated markerless deletions of the *comM* gene (Δ*comM*) in both *A. baumannii* clinical
235 strains. Consistently, deletion of the *comM* gene in both strains resulted in transformation
236 levels comparable to levels observed for the wild type strains bearing a genuine insertion in
237 the *comM* gene by the AbaR island (Fig. 3A and B). Thus, prior to acquisition of AbaR, both
238 strains were transformable at higher levels, and the subsequent disruption generates a
239 transformation defects that is consistent with previous observations made in other species or
240 genus (10,11). The data show that the AbaR-inactivated *comM* gene, once cured of AbaR, is
241 still functional. This suggests that it has not been affected by mutational drifts, confirming that
242 acquisition of AbaR occurred relatively recently (22).
243 The fact that acquisition of the AbaR element in the *comM* gene alters the natural
244 transformability of *A. baumannii* and its capacity for further acquisitions by natural
245 transformation is intriguing. The significance of lower transformability is not known but
246 theoretical work suggest that intermediate transformation rates allows bacteria to maximize
247 fitness in fluctuating, stressful environments such as repeated antibiotic stress (25). Our
248 results then suggest that acquisition of AbaRs, and associated resistance, provide a short-
249 term benefit in environment with constant exposure to antibiotics but may ultimately lead to an
250 evolutionary dead end.
251
252 **CONCLUSION**
253 By exploiting natural transformation, we setup a genetic system to easily edit the genome of
254 *A. baumannii*. We used this strategy to perform small gene deletion but also to cure large
255 genomic islands in two MDR clinical *A. baumannii* strains. Moving beyond predicted
256 resistance, this system allowed experimental determination of the antibiotic resistance
257 conferred by the islands. We conclude that acquisition of AbaR islands may increase *A.*
258 *baumannii* strains resistance to antibiotics but it comes at the cost of decreasing the cell
259 ability to perform natural transformation by disrupting the *comM* gene. Thus, acquisition of
260 AbaR may impact genome evolution of *A. baumannii*. From an applied perspective, the

261 simultaneous deletion of AbaR and restoration of the *comM* function in two clinically relevant
262 strains made them even more genetically amenable, blazing the trail for mutational analysis of
263 these model *A. baumannii* strains.
264
265
266
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276
277
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354

1 **Table 1.** Antimicrobial resistance profiles of *A. baumannii* strains AB5075 and AYE deleted of
2 their AbaR island (-T) in comparison to the wild type strains (WT).
3

Antibiotics[a]	Inhibition zone diameter in mm (category[b])			
	AB5075 WT	AB5075-T	AYE WT	AYE-T
AMK	20 (S)	20 (S)	8 (R)	21 (S)
GEN	11 (R)	12 (R)	6 (R)	19 (S)
TOB	10 (R)	10 (R)	6 (R)	18 (S)
CIP	6 (R)	6 (R)	6 (R)	6 (R)
PIP	6 (R)	6 (R)	6 (R)	6 (R)
TZP	6 (R)	6 (R)	14 (R)	15 (R)
TIC	6 (R)	6 (R)	6 (R)	19 (I)
TIM	6 (R)	6 (R)	15 (I)	18 (I)
CAZ	6 (R)	6 (R)	6 (R)	11 (R)
FEP	6 (R)	6 (R)	6 (R)	16 (I)
IMP	6 (R)	6 (R)	28 (S)	28 (S)
MEM	ND	ND	20 (S)	22 (S)
ATM	6 (R)	6 (R)	6 (R)	11(R)
SUF	6 (R)	6 (R)	6	23
TET	18 (S)	18 (S)	ND	ND
TET (E-test)[c]	ND	ND	128	8

4
5 [a] Abbreviations. AMK: Amikacin, GEN: Gentamicin, TOB: Tobramycin, CIP: Ciprofloxacin, PIP:
6 Piperacillin, TZP: Piperacillin + tazobactam, TIC: Ticarcillin, TIM: TIC + clavulanic acid, CAZ:
7 Ceftazidime, FEP: Cefepime, IMP: Imipenem, MEM: Meropenem, ATM: Aztreonam, TET:
8 Tetracycline, SUF: sulfonamide.
9 [b] Categorization (R: resistant, S: susceptible) is detailed in the materials and methods section.
10 [c] E-test strips were used to determine the MICs of tetracycline for the strains AYE and AYE-T.
11 ND: Note Done.
12

355 ***Figure legends***
356
357 **Figure 1. PCR-based genome editing of transformable *A. baumannii***
358 Schematic representations of steps for gene inactivation (dark blue gene) using natural
359 transformation (A) Annealing sites for primers of PCR assemblies on their respective
360 templates: either *A. baumannii* genomic DNA for amplifications of 2 kbp upstream and
361 downstream of the deleted region (Δ) or the laboratory plasmid pMHL-2 that bears the
362 counter-selectable cassette (light blue) with a *sacB* gene conferring sucrose sensitivity and
363 the *aac(3)IV* gene encoding for resistance to apramycin. Note that primers P2, P3 and P7
364 share annealing sequences with primers P5, P6 and P8 respectively. (B) Chimeric PCR
365 product (orange) obtained after two steps PCR assembly of products [P1 + P2] with [P5 + P6]
366 and [P3 + P4]. This chimeric product bears the *sacB_aac* cassette flanked by two 2 kbp-long
367 regions of homology with the recipient cell's genome (grey areas). These homologous regions
368 allow the recombination of the chimeric PCR product into the recipient cell's genome
369 resulting, upon apramycin selection of the transformants, in the intermediary genotype
370 represented in C. (C) Chimeric PCR product obtained after two steps assembly PCR of
371 products [P1 + P8] with [P7 + P4] bearing the deleted gene flanked by homologous regions
372 (grey areas) allowing recombination and replacement of the counter-selectable cassette in the
373 intermediary recipient genome. The transformants are selected based on their sucrose
374 resistance resulting in the final scarless edited genome (D).
375
376 **Figure 2.** Genetic engineering of AbaR curing in *A. baumannii*
377 Schematic representations of (A) AbaR11 inserted in the *comM* gene in *A. baumannii* AB5075
378 genome. All the sequences and genes in grey color belong to the wild type AB5075
379 chromosome, the interrupted *comM* gene is however highlighted in dark blue. For the AbaR
380 island, modules are labeled following previous convention (9). For illustration purposes, scale
381 of the AbaR island is not respected (B) Intermediary genetic construct with the counter-
382 selectable cassette replacing the AbaR11 island. (C) Final genetic structure of the locus after
383 restoring the *comM* gene integrity.
384
385 **Figure 3.** Transformation efficiency of *A. baumannii* strains AB5075 (A) and AYE (B) of a 3,8
386 kbp-long PCR product carrying a mutation in the *rpoB* gene conferring resistance to
387 rifampicin. For both strains, either the wild type, their "T" (ΔAbaR / repaired *comM*) or Δ*comM*

388 derivatives were tested. At least seven independent transformation assays were performed
389 on at least two separate occasions represented by separate dots and corresponding boxplots.
390 All results were above the limit of detection (10^{-8}). Pairwise comparisons using the non-
391 parametric Mann-Whitney-Wilcoxon test (two tailed) gave at least *p*-values <0.005 between
392 transformation efficiencies obtained for the wild type/Δ*comM* strains and the T-derivatives.

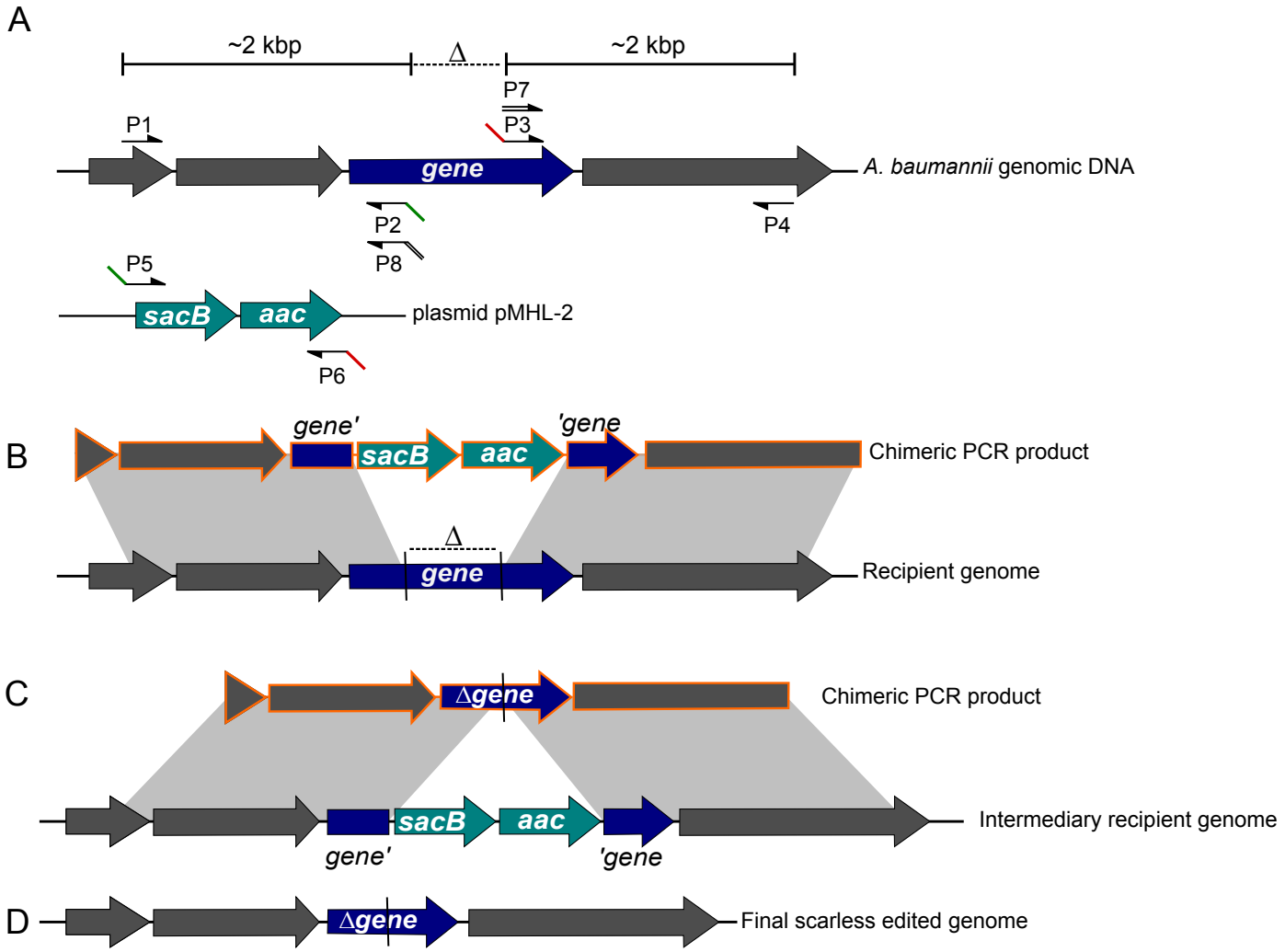

Figure 1. PCR-based genome editing of transformable *A. baumannii*

Schematic representations of steps for gene inactivation (dark blue gene) using natural transformation (A) Annealing sites for primers of PCR assemblies on their respective templates: either *A. baumannii* genomic DNA for amplifications of 2 kbp upstream and downstream of the deleted region (Δ) or the laboratory plasmid pMHL-2 that bears the counter-selectable cassette (light blue) with a *sacB* gene conferring sucrose sensitivity and the *aac(3)IV* gene encoding for resistance to apramycin. Note that primers P2, P3 and P7 share annealing sequences with primers P5, P6 and P8 respectively. (B) Chimeric PCR product (orange) obtained after two steps PCR assembly of products [P1 + P2] with [P5 + P6] and [P3 + P4]. This chimeric product bears the *sacB_aac* cassette flanked by two 2 kbp-long regions of homology with the recipient cell's genome (grey areas). These homologous regions allow the recombination of the chimeric PCR product into the recipient cell's genome resulting, upon apramycin selection of the transformants, in the intermediary genotype represented in C. (C) Chimeric PCR product obtained after two steps assembly PCR of products [P1 + P8] with [P7 + P4] bearing the deleted gene flanked by homologous regions (grey areas) allowing recombination and replacement of the counter-selectable cassette in the intermediary recipient genome. The transformants are selected based on their sucrose resistance resulting in the final scarless edited genome (D).

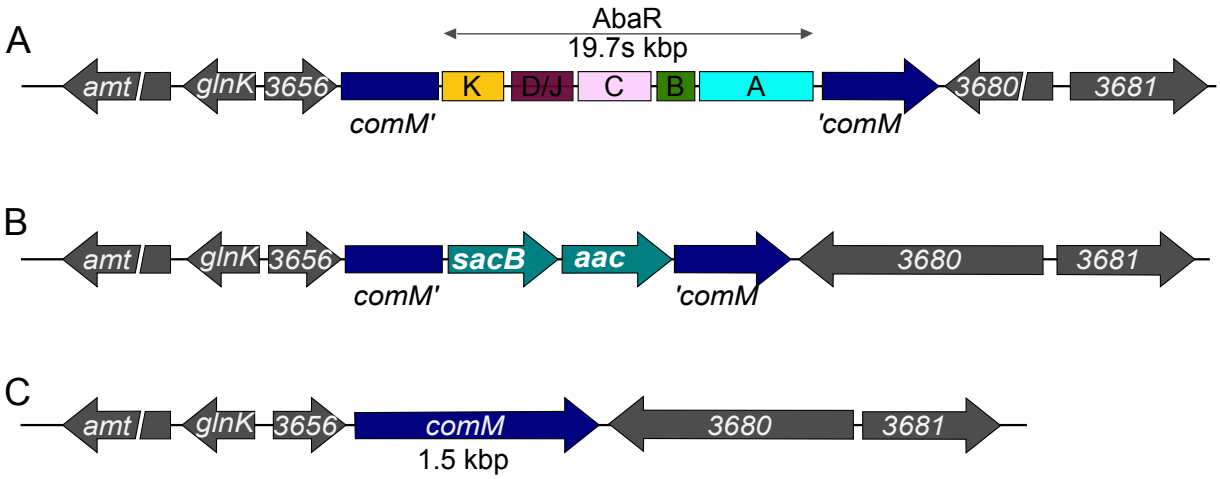

Figure 2. Genetic engineering of AbaR curing in *A. baumannii.*
Schematic representations of (A) AbaR11 inserted in the *comM* gene in *A. baumannii* AB5075 genome. All the sequences and genes in grey color belong to the AB5075 WT chromosome, the interrupted *comM* gene is however highlighted in dark blue. For the AbaR island, modules are labeled following previous convention (9). For illustration purposes, scale of the AbaR island is not respected (B) Intermediary genetic construct with the counter-selectable cassette replacing the AbaR11 island. (C) Final genetic structure of the locus after restoring the *comM* gene integrity.

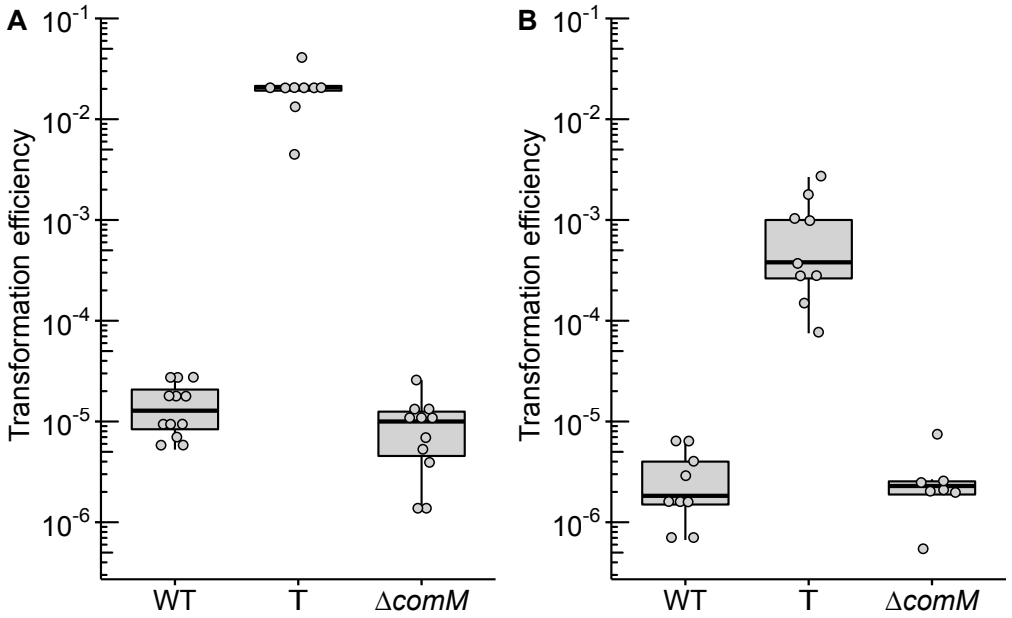

Figure 3. Transformation efficiency of *A. baumannii* strains AB5075 (A) and AYE (B) of a 3,8 kbp-long PCR product carrying a mutation in the *rpoB* gene conferring resistance to rifampicin. For both strains, either the wild type, their "T" (ΔAbaR / repaired *comM*) or Δ*comM* derivatives were tested. At least seven independent transformation assays were performed on at least two separate occasions represented by separate dots and corresponding boxplots. All results were above the limit of detection (10^{-8}). Pairwise comparisons using the non-parametric Mann-Whitney-Wilcoxon test (two tailed) gave at least *p*-values <0.005 between transformation efficiencies obtained for the wild type/Δ*comM* strains and the T-derivatives.